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10/573,813	07/27/2006	Mitsufumi Wada	1034232-000019	9341

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EXAMINER	
LEAVITT, MARIA GOMEZ	
ART UNIT	**PAPER NUMBER**
1633	

NOTIFICATION DATE	DELIVERY MODE
08/26/2009	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

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Office Action Summary	Application No.	Applicant(s)	
	10/573,813	WADA ET AL.	
	Examiner	Art Unit	
	MARIA LEAVITT	1633	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *22 May 2009*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *7,15,16,18,19,22 and 41-75* is/are pending in the application.

4a) Of the above claim(s) *22 and 46-75* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *7,15,16,18,19 and 41-45* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *28 March 2006* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *03-28-2006*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

Detailed Action

Applicants' responses of 02-23-2009 and 05-22-2009 to the restriction requirements of 01-26-2009 have been entered.

Claim status. Claims 7, 15, 16, 18, 19, 22, 41-75 are currently pending. Claims 7, 15, 18 and 22 have been amended, claims 1-6, 8-14, 17, 20, 21 and 23-40 have been cancelled, and claims 41-75 have been added by Applicants' amendment filed on 02-23-2009.

Applicants' election of Group III in the office action mailed on 01-26-2009, original claims 7-9 (claims 8 and 9 are now cancelled), drawn to a **microorganism** in which activity of FAD-dependent D-lactate dehydrogenase (dld) inherent in the microorganism is inactivated or decreased, activity of pyruvate formate-lyase (pfl) is inactivated or decreased, and activity of *Escherichia coli*-derived NADH-dependent D-lactate dehydrogenase is enhanced is acknowledged. New claims 46-75 are methods claims that depend directly or indirectly from product claim 7. For example, claim 64, drawn to a method for producing lactic acid, depends from method claim 58 which depends from method claim 47 which depends from the elected invention, i.e. claim 7.

Response to arguments

At pages 8 and 9 of the remarks filed on 02-23-2009, Applicants essentially argue that when Group III is examined, at least Groups VI and VII should be rejoined and examined in the same application, in part, because the promoter specified in these Groups relates to enhancement of the activity of *Escherichia coli*-derived NADH-dependent ldhA. The above arguments have been fully considered and deemed persuasive. Accordingly, the restriction requirements among Groups VI, i.e. claim 15, and VII, i.e., 18 and 19, have been withdrawn.

However, the requirement for restriction between Groups I, II, IV and V and VIII is maintained for reasons of record as set forth in the office action of 01-26-2009. Accordingly, Claims 22 corresponding to original Group VIII, and method claims 46 to 75, corresponding to original Groups I, II, IV and V (now cancelled) have been withdrawn for further examination pursuant to 37 CFR 1.142(b), as being drawn to a nonelected invention, there being no allowable generic or linking claim.

Please note that the MPEP 1893.03(d) states:

> If an examiner (1) determines that the claims lack unity of invention and (2) requires election of a single invention, when all of the claims drawn to the elected invention are allowable (i.e., meet the requirements of 35 U.S.C. 101, 102, 103 and 112), the nonelected invention(s) should be considered for rejoinder. Any nonelected product claim that requires all the limitations of an allowable product claim, and any nonelected process claim that requires all the limitations of an allowable process claim, should be rejoined. See MPEP § 821.04 and § 821.04(a). Any nonelected processes of making and/or using an allowable product should be considered for rejoinder following the practice set forth in MPEP § 821.04(b).

The requirement is still deemed proper and made Final.

Also note that after a final requirement for restriction, the Applicants, in addition to making any response due on the remainder of the action, may petition the Commissioner under 37 CFR 1.144 to review the requirement. Petition may be deferred until after final action on or allowance of claims to the invention elected, but must be filed not later than appeal. A petition will not be considered if reconsideration of the requirement was not requested. (See § 1.181.).

Accordingly claims 7, 15, 16, 18, 19 and 41-45 are examined on the merits to which the following grounds of rejection are applicable.

Title of the Invention

The title of the invention is not descriptive. The title uses parentheses to comments on or qualifies part of the sentences. It is unclear whether the limitations in parentheses are meant to be part of the title or whether they are only suggestions/examples. A new title is required that is clearly indicative of the invention to which the claims are directed.

Information Disclosure Statement

The listing of references in the specification at pages 12-13 is not a proper information disclosure statement. 37 CFR 1.98(b) requires a list of all patents, publications, or other information submitted for consideration by the Office, and MPEP § 609.04(a) states, "the list may not be incorporated into the specification but must be submitted in a separate paper." Therefore, unless the references have been cited by the examiner on form PTO-892, they have not been considered.

Claim Rejections - 35 USC § 112- Second Paragraph

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 18 and 19 are rejected under 35 U.S.C. **§** 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 18 and 19 are indefinite in their recitation of the phrases “a gene encoding the Escherichia coli-derived NADH-dependent D-lactate dehydrogenase” and “ is a promoter of an *E. coli*-derived glyceraldehyde-3-phophate dehydrogenase gene”, respectively. It is unclear the nature and number of steps required to obtain a "derivative" of an *E. coli*-derived NADH-

dependent D-lactate dehydrogenase and a glyceraldehyde-3-phophate dehydrogenase gene promoter of an *E. coli*. The term "derivative" implies a number of different steps that may or may not result in a change in the functional characteristics of a NADH-dependent D-lactate dehydrogenase and a glyceraldehyde-3-phophate dehydrogenase gene promoter from the source that it is "derived from". It would be remedial to amend the claim language to use the term "obtained from", which implies a more direct method of acquiring the *E. coli* genes.

Claim Rejections - 35 USC § 103

To the extent that the claimed invention embraces a microorganism in which the activity of FAD-dependent D- lactate dehydrogenase (*dld*) inherent in the microorganism is decreased, activity of pyruvate formate-lyase (*pfl*) inherent in the microorganism is inactivated or decreased, and activity of *Escherichia coli*-derived NADH-dependent D-lactate dehydrogenase (*ldhA*) inherent in the microorganism is enhanced, the following rejections are applicable.

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later

invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 7, 15, 16, 18, 42 and 44 are rejected under 35 USC 103 as being unpatentable over Zhou et al., (Jan. 2003, Applied and Environmental Microbiology, pp. 399-407, or record) in view of Yang et al., (1999, Metabolic Engineering, pp. 141-152 , of record) as evidenced by Bunch et al., (Microbiology 1997, pp.187-195, of record) and further in view of Shaw et al., (1975, J. Bacteriology, pp. 1047-1975).

Zhou et al., discloses various mutant of *E. coli* (i.e., W3110) characterized in that they produce D-lactic acid. Specifically, Zhou teaches genetically engineers E. *coli* wherein the activity of pyruvate formate-lyase (pfl) inherent in the microorganism is inactivated by mutation of the *pftB* gene (page 400, col. 2, paragraph 2), which reduces the catabolism of pyruvate to formate (page, 401, Fig. 1). Zhou et al., describes that this single mutation eliminates the production of formate, ethanol and acetate in tube cultures containing 1% glucose (p. 402, col. 2). In addition, Zhou et al., teaches additional mutations in the bacterial genome responsible for the catabolism of pyruvate including inactivation of genes encoding for alcohol /aldehyde dehydrogenase (*adhE*) which eliminates or reduces ethanol production and acetate kinase (*ackA*) which eliminates or reduces acetate production, e.g., SZ63 (*pflB frd adlhE ackA*) (p. 403, table 3). Note that by eliminating genes in the microorganism responsible for the catabolism of pyruvate, then pyruvate accumulates. Also note that glycolysis terminates when pyruvate is reduced via NADH and H+ to lactate (e.g., in the presence of lactate dehydrogenase, e.g *ldh*), or in the presence of alcohol dehydrogenase ethanol is formed, or in the presence of pyruvate formate lyase (e.g., in the presence of *pfl*), fermentation terminates with the production of

acetate, ethanol, and formate, or hydrogen plus carbon dioxide (page 401, col. 2, last paragraph; Fig. 1)(**Current claims 7, 16, 42, and 44 in part**).

Zhou et al., does not specifically teach the metabolic engineering *E. Coli* with reduced activity in FAD-dependent D- lactate dehydrogenase (dld) and wherein activity of *Escherichia coli*-derived NADH-dependent D-lactate dehydrogenase (ldhA) inherent in the microorganism is enhanced.

However, at the time the invention was made, Yang discloses the amplification of the lactate flux resulting from overexpression of fermentative lactate dehydrogenase (LDH) which catalyzes the formation of lactate by transforming *E. coli* with a plasmid carrying the NADH-dependent D-lactate dehydrogenase (ldhA) gene. Moreover, Yang teaches that LDH activities of strains carrying plasmid pTY2 showed a higher LDH activity, e.g., 25.1, vs. control values of 0.63 (p. 143, col. 1, paragraph 1; table 3). The amount of lactate produced by *E. Coli* strain YBS132 (*ackA-pta-ldhA*-) *ldhA* plasmid was 6.91 in relation to parental production of strain YBS132 (*ackA-pta-ldhA*-) (page 147, Table 5), clearly indicating that lactate productivity is improved by addition of the plasmid encoding the *ldhA* gene . Furthermore, Yang teaches plasmid pFB15 a multicopy vector carrying the ldhA gene under the control of the ldhA promoter as evidenced by Bunch (p. 142, col. 2, last paragraph; see also Bunch for multicopy cloning vector of the ldhA gene; p. 190, Fig. 1; p. 193, col. 1, paragraph 1) (**Current claims 7, 15, 16 and 18, in part**).

The combined disclosure of Zhou et al. and Yang fails to teach reduced activity in FAD-dependent D- lactate dehydrogenase (dld).

However, at the time the invention was made Shaw et al., characterizes double mutants *Escherichia coli* strains JS150 and JS151 lacking L and D-lactate dehydrogenases. Moreover, Shaw discloses that mutants for both membrane bound L and D-lactate dehydrogenases in *E. Coli* are unable to oxidize 2-hydroxy-3-butenoic acid (Vinylglycolic). Table 2, indicates that L and D-lactate dehydrogenases are differentially expressed in *E. coli* strains. Though Shaw, et al. does not specifically discloses how in JS150 and JS151 both L and D-lactate dehydrogenases regulate the metabolism of pyruvate to D-lactate, it would have been *prima facie* obvious for one of ordinary skill in the art with the aim of enhancing production of D-lactic acid, to metabolically engineered *E. coli* in an attempt to study whether the flux from pyruvic acid to lactate is modified (e.g., enhanced or reduced) by each L and D- membrane bound flavoproteins lactate dehydrogenases. **(Current claims 7 and 16, in part)**.

Therefore, in view of the benefits of metabolic engineering of *E. Coli* for enhanced production of D-lactic acid based on the generation of the mutant *E. Coli* wherein the activity of pyruvate formate-lyase (pfl) inherent in the microorganism is inactivated by mutation of the *pftB* gene as taught by Zhou, it would have been *prima facie* obvious to one of ordinary skill in the art with the aim of further enhancing production of D-lactic acid to inactivate or overexpress other genes responsible for metabolizing glucose to D-lactic acid including overexpressing NADH-dependent D-lactate dehydrogenase (*ldhA*) gene as taught by Yang. Metabolic engineering is a complex science but with well-established protocols for genetic manipulation and large physiological knowledge of fermentative pathways in *E. coli* for the production of D-lactate. Moreover, it would have been *prima facie* obvious in an attempt to provide enhanced production of D-lactic acid, to metabolically engineered E. *coli* to study how the flux from pyruvic acid to

lactate is modified (e.g., enhanced or reduced) by each L and D-lactate dehydrogenases membrane-bound flavoproteins as taught by Shaw et al., and the NADH-dependent D-lactate dehydrogenase disclosed by Yang as the cumulative effect of multiple mutations may be negative, additive or synergistic and NADH-linked lactate dehydrogenase (LDH) was well known in the art to catalyzed the conversion of pyruvate to lactate. Additionally, a person with ordinary skills has good reason to pursue the known options within his or her technical grasp. Moreover, based on the detailed teachings of the Zhou, the Yang and Shaw teachings and the high level of skill in the art of molecular cloning, the skilled artisan would have a reasonable expectation of success in generating a transformed *E. coli* strain in which the activity of FAD-dependent D- lactate dehydrogenase (dld) inherent in the microorganism is decreased, activity of pyruvate formate-lyase (pfl) inherent in the microorganism is decreased, and activity of *Escherichia coli*-derived NADH-dependent D-lactate dehydrogenase (ldhA) inherent in the microorganism is enhanced resulting in improvement of D-lactic acid production.

Claims 41, 43 and 45 are rejected under 35 USC 103 as being unpatentable over Zhou et al., (Jan. 2003, Applied and Environmental Microbiology, pp. 399-407, or record) in view of Yang et al., (1999, Metabolic Engineering, pp. 141-152 , of record) as evidenced by Bunch et al., (Microbiology 1997, pp.187-195, of record) and Shaw et al., (1975, J. Bacteriology, pp. 1047-1975) as applied to claims 7, 15, 16, 18, 42 and 44 above, and further in view of Courtright et al., (*J Bacteriol.* 1970, pp. 722–728, of record)

The teachings of Zhou, Yang and Shaw are outlined in the paragraphs above.

The combined disclosure of Zhou, Yang and Shaw fails to teach a microorganism wherein at least one of the activity of malate dehydrogenase (*mdh*) inherent in the microorganism and activity of aspartate ammonia-lyase (*aspA*) inherent in the microorganism are inactivated or decreased.

However, at the time the invention was made, Courtright discloses *Escherichia coli* K-12 mutants devoid of malate dehydrogenase activity. Courtright discloses two pathways to produce succinic acid under anaerobic conditions, i.e., a pathway to lead to succinic acid via malic from oxalacetic acid, and a pathway to lead to succinic acid via aspartic acid from oxalacetic acid (page 727, Fig. 1, legend; p. 726, col. 1, paragraph 1). Moreover, Courtright teaches that malate dehydrogenase (mdh) mutants do not require succinate for anaerobic growth on glucose (e.g., there is not activity from oxalacetate to malic acid under anaerobic conditions) and that mutants devoid of malate dehydrogenase activity activate the pathway leading to succinic acid via aspartic acid from oxalacetic acid.

Therefore, it would have been *prima facie* obvious to one of ordinary skill in the art in an attempt to provide enhanced production of D-lactic acid, to metabolically engineered E. *coli* generated y the combined teachings of Zhou, Yang and Shaw to further reduce malate dehydrogenase activity, particularly because Courtright discloses *Escherichia coli* K-12 mutants devoid of malate dehydrogenase activity (*mdh*) do not require succinate for anaerobic growth on glucose and pyruvate serves as a precursor of OAA and Lactate. Thus by inhibiting the pyruvate formate lyase, then pyruvate is redirected to lactate formation. The manipulation of previously identified and well-established protocols for generation of metabolically engineered E. *Coli* is within the ordinary level of skill in the art of biotechnological fermentation products. Thus, the

skilled artisan would have a reasonable expectation of success in generating a metabolically transformed *E. coli* strain in which the activity of FAD-dependent D- *dld* inherent in the microorganism is decreased, activity of *pfl* inherent in the microorganism is decreased, activity of *Escherichia coli*-derived NADH-dependent D-*ldhA* inherent in the microorganism is enhanced and activity of *mdh* is reduced resulting in improvement of D-lactic acid production.

Claim 18 and 19 are rejected under 35 USC 103 as being unpatentable over Zhou et al., (Jan. 2003, Applied and Environmental Microbiology, pp. 399-407, or record) in view of Yang et al., (1999, Metabolic Engineering, pp. 141-152 , of record) as evidenced by Bunch et al., (Microbiology 1997, pp.187-195, of record) and Shaw et al., (1975, J. Bacteriology, pp. 1047-1975) as applied to claims 7, 15, 16, 18, 42 and 44 above, and further in view of Maier et al (US Patent Application No. 10/620487, Date of filing July 16, 2003)

The teachings of Zhou, Yang and Shaw are outlined in the paragraphs above.

The combined disclosure fails to teach a glyceraldehyde-3-phosphate dehydrogenase promoter from *E. coli* .

However, at the time the invention was made, Maier et al., discloses genetically engineered microorganism including transformant *Escherichia coli* (page 2, paragraph [0026]) for producing amino acids and amino acid derivatives of the phosphoglycerate family. Moreover, amino acid-overproducing microorganisms include cloning of a yfiK gene into plasmid vectors under the control of suitable promoters for overexpression of the yfiK-gene product (page 2, [0027][0028]) . Maier teaches suitable promoters including "the constitutive GAPDH promoter of the gapA gene or the inducible *lac, tac, trc, lambda, ara* or *tet* promoters in *Escherichia coli*

are known to the skilled worker" (page 2 [0031],) [0035]). Furthermore, Maier successfully exemplifies expression of the yfiK gene product in transformant E. coli driven by the glyceraldehyde-3-phosphate dehydrogenase promoter from *E. coli* (page 3, paragraph [0065]).

The manipulation of previously identified DNA fragments and cell transformation systems is within the ordinary level of skill in the art of molecular biology. Therefore, it would have been *prima facie* obvious for one of ordinary skill in the art at the time the invention was made, on teachings provided by the combined cited references, to substitute any of the known promoters of *E. coli* as taught by Maier including a glyceraldehyde-3-phosphate dehydrogenase promoter in the recombinant plasmid pFB15 taught by Yang et al., for overexpression of a gene of interest with a reasonable expectation of success, particularly because Maier exemplifies successfully exemplifies expression of the yfiK gene product in transformant E. coli driven by the glyceraldehyde-3-phosphate dehydrogenase promoter from *E.* coli to enhance production of amino acid in a fermentation process.

Conclusion

Claims 7, 15, 16, 18, 19 and 41-45 are not allowable.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Maria Leavitt whose telephone number is 571-272-1085. The examiner can normally be reached on M-F.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Joseph Woitach, Ph.D can be reached on (571) 272-0739. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

To aid in correlating any papers for this application, all further correspondence regarding his application should be directed to Group Art Unit 1633; Central Fax No. (571) 273-8300. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to (571) 272-0547.

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/Maria Leavitt/

Maria Leavitt, PhD
Primary Examiner, Art Unit 1633